

*Communications to the Editor***Spectroscopy and Electrochemistry of Ruthenium-Modified Nucleic Acids: Design of a Novel Metal-Binding Nucleoside**

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Received November 10, 1999

Electron transfer (ET) reactions through DNA¹ have been the subject of numerous investigations due to the implications for light-induced DNA damage² and the quest for understanding long-range ET events in biological molecules.³ An important objective in this area continues to be the facile and site-specific incorporation of metal complexes into DNA. While recent work has focused on nucleobasic and nonnucleosidic sites for the attachment of high-potential complexes,⁴ our efforts have concentrated on the ribose ring (to minimize structural perturbations) as the incorporation site for both high- and low-potential metal complexes.⁵

To this end, we have designed a novel chelating nucleoside (**1**) that enables the preparation of a series of metallonucleosides. We report the synthesis and spectral characteristics of a unique electron donor–acceptor pair prepared from **1** consisting of low-(**2**) and high-potential (**3**) metallonucleosides. Further, we describe the first example of a metal-containing oligonucleotide (**4**) prepared by solid-phase methods starting with the metal complex derivatized directly to a solid support.

The metal-binding nucleoside **1**, 5'-O-(4,4'-dimethoxytrityl)-2'-iminomethylpyridyl-2'-deoxyuridine, was readily prepared in situ by condensation of 5'-DMT protected 2'-amino-2'-deoxyuri-

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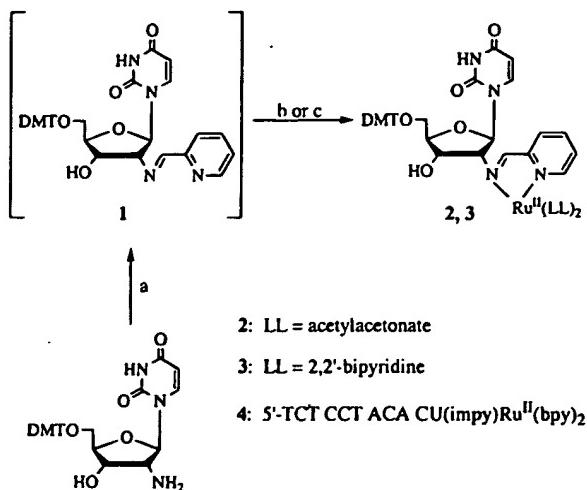


Figure 1. Synthesis and structures of 2'-ruthenated nucleosides and oligonucleotide **4**: (a) 2-pyridinecarboxaldehyde, EtOH, 2 h; (b) Ru(acac)₂(CH₃CN)₂, EtOH, 1 h, 79% yield; and (c) Ru(bpy)₂Cl₂, EtOH, 4 h, 19% yield.

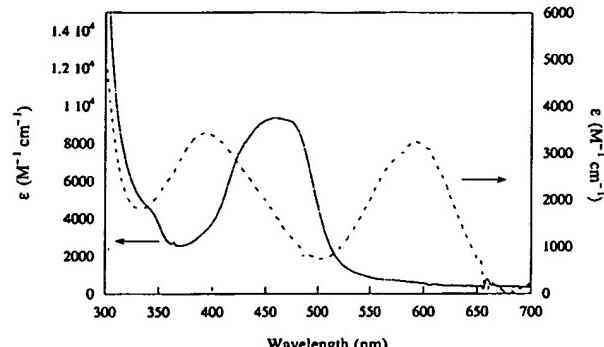


Figure 2. Absorption spectra of [Ru(acac)₂(**1**)] (**2**) (dashed line) and [Ru(bpy)₂(**1**)]²⁺ (**3**) (solid line) in ethanol and methanol, respectively.

dine⁶ with 2-pyridinecarboxaldehyde (Figure 1).⁷ The ruthenated nucleosides **2** and **3** were prepared by subsequent addition of Ru(acac)₂(CH₃CN)₂⁸ (acac = acetylacetone) and Ru(bpy)₂Cl₂ (bpy = 2,2'-bipyridine) to **1**; they represent the range of metal complexes that can be inserted at the 2'-chelate site.

Metallonucleoside **3** was selected for derivatization to the solid support due to its stability in both the mildly acidic and strongly basic solutions that are routinely encountered during automated DNA synthesis. Treatment of **3** with succinic anhydride produced the hemisuccinate form that was isolated by flash chromatography in 54% yield.⁹ Derivatization of the solid support with succinated **3** enabled the preparation of oligonucleotide **4** with yields

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comparable to those observed in automated DNA synthesis. The duplex formed with **4** and its complementary sequence strand exhibited a single, cooperative melting transition with $T_m = 50^\circ\text{C}$ (50 mM NaPi, 0.5 M NaCl, pH 7.0; 2 °C higher than T_m of the corresponding unmodified duplex).

The spectral characteristics of metallonucleosides **2** and **3** are shown in Figure 2. The absorption spectrum of **2** reveals maxima at 396 ($\epsilon = 3600 \text{ M}^{-1} \text{ cm}^{-1}$) and 592 nm ($\epsilon = 3400 \text{ M}^{-1} \text{ cm}^{-1}$) in EtOH, which shift slightly in CH_2Cl_2 (392 nm, 3600 $\text{M}^{-1} \text{ cm}^{-1}$; 602 nm, 3700 $\text{M}^{-1} \text{ cm}^{-1}$). The reduction potentials of **2** and the model complex, $\text{Ru}(\text{acac})_2(\text{impy})$ (impy = iminomethylpyridine), are 92 and 33 mV vs Ag/AgCl, respectively. Apparently, the proximity of the nucleoside to the metal center causes the reduction potential to shift to more positive values.

The electronic spectra of **3** and **4** display a broad absorption band at 480 nm ($\epsilon = 9100 \text{ M}^{-1} \text{ cm}^{-1}$), consistent with that previously reported by Keene and Meyer for $\text{Ru}(\text{bpy})_3(\text{impy})$.¹⁰ This band, which is red-shifted from $\lambda_{\text{max}} = 452 \text{ nm}$ ($\epsilon = 14600 \text{ M}^{-1} \text{ cm}^{-1}$) for $[\text{Ru}(\text{bpy})_3]^{2+}$, reveals the effect of substituting iminomethylpyridine for bipyridine. Electrochemical measurements on **3** (in dichloromethane) and **4** (in water) give Ru(III/II) reduction potentials of 1.4 and 1.1 V vs Ag/AgCl, respectively.

Irradiation of **3** and **4** at 480 nm yields identical lifetimes ($\lambda_{\text{max}}(\text{em}) = 740 \text{ nm}$; $\tau = 42 \text{ ns}$; $\Phi = 1.1 \times 10^{-4}$), which are shorter than that observed for $[\text{Ru}(\text{bpy})_3]^{2+}$ ($\lambda_{\text{max}}(\text{em}) = 625 \text{ nm}$; $\tau = 620 \text{ ns}$; $\Phi = 0.042$).¹¹ Indeed, the differences in lifetime and quantum yield correspond to an increase in the nonradiative rate constant (k_{nr}) from $1.54 \times 10^6 \text{ s}^{-1}$ for $[\text{Ru}(\text{bpy})_3]^{2+}$ to $2.38 \times 10^7 \text{ s}^{-1}$ for **4**. The radiative rate constant (k_r) for **4** is on the order of 10^3 s^{-1} , suggesting that the emissive state is similar to other $\text{Ru}(\text{bpy})_3^{2+}$ -type chromophores. In comparison to $[\text{Ru}(\text{bpy})_3]^{2+}$, the iminomethylpyridine ligand in $[\text{Ru}(\text{bpy})_2(\text{impy})]^{2+}$ is responsible for the red-shifted emission and the increase in k_{nr} .

We utilized resonance Raman (rR) spectroscopy to investigate the nature of the visible transitions of these complexes. The rR spectrum of **4** (identical to **3**; 441.6 nm excitation) is similar to that of other ruthenium polypyridyl compounds (Figure 3).¹² The peaks at 1023, 1173, 1276, 1316, 1488, 1552, and 1604 cm^{-1} correspond well to those observed in the rR spectrum of $[\text{Ru}(\text{bpy})_3]^{2+}$.¹³ Additional peaks (1242, 1471 cm^{-1}) represent excited-state distortions that are not present in $[\text{Ru}(\text{bpy})_3]^{2+}$, and can be attributed to the impy ligand.

Despite the weak absorption of **2** at the available excitation frequencies (441.6 and 514.5 nm), rR spectroscopy provided valuable data. The rR spectrum (441.6 nm excitation) of **2** reveals

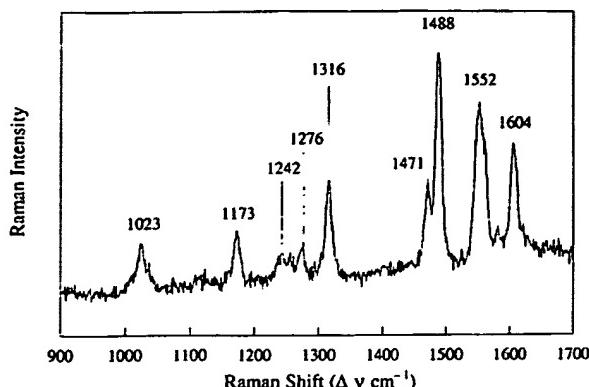


Figure 3. Resonance Raman spectrum of oligonucleotide **4** in unbuffered aqueous solution.

characteristic acac vibrations at 1528 ($\nu(\text{CO})$) and 1234 cm^{-1} ($\nu(\text{C}-\text{C})$).^{14,15} Spectral features typically seen for polypyridyl complexes are absent, confirming the Ru $d\pi \rightarrow \text{acac} \pi^*$ nature of the transition.¹⁶ The spectrum obtained with 514.5 nm excitation yields vibrations similar to those observed for complex **4** (1249, 1288, 1501, 1530, 1551, and 1597 cm^{-1}). As a result, the low-energy band at 592 nm is assigned as a Ru $d\pi \rightarrow \text{impy} \pi^*$ transition.

The synthesis of both the low-potential complex **2** and the metal-modified solid support represents a significant advance in the development of metal-containing oligonucleotides. The synthetic versatility of **1** is demonstrated by the preparation of **2** and **3**. Further, the distinct absorption and electrochemical features of these complexes are well-suited for ground-state ET studies involving DNA. Future work will examine these characteristics and focus on additional means of incorporating similar oxidants.

Acknowledgment. We wish to thank Jay R. Winkler and Harry B. Gray of the Beckman Institute Laser Resource Center (BILRC) and Graham Heath of the Australian National University for helpful discussions. Mass spectrometry results were provided by Gary Hathaway (Caltech PPMAL). This work was supported by NIST (ATP) Award 70NANB5H1031, Jet Propulsion Laboratory (JPL) 67192, and the Beckman Institute Biological Imaging Center.

Supporting Information Available: Instrumental details and experimental procedures for complexes **1–4** and $\text{Ru}(\text{acac})_2(\text{impy})$ (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Education

Postdoctoral Research Fellow. California Institute of Technology, 1987-89.
Advisor: Professor Harry B. Gray.

National Institutes of Health Postdoctoral Fellow. Harvard Medical School and the Massachusetts General Hospital, 1985-1987.
Advisor: Professor Thomas J. Brady.

Ph.D. in Inorganic Chemistry, The Ohio State University; Columbus, Ohio, August, 1985. Dissertation Advisor: Professor Daryle H. Busch.

M.S. in Chemistry (Division of Biochemistry), The Ohio State University, Columbus Ohio, 1982. Thesis Advisor: Professor Perry A. Frey.

B.S. with Honors in Chemistry, Arizona State University; Tempe Arizona, May, 1980. Honors Thesis Advisor: Professor Therald Moellar.

Professional Experience

Senior Research Faculty, Division of Biology and the Beckman Institute, California Institute of Technology, (1996-present).

Director: Program for Bioinorganic Drug Design and Discovery, Beckman Institute, California Institute of Technology, (1993-present).

Research Faculty: Division of Biology, California Institute of Technology, (1991-96).

Research Interests

Chemistry of Life Sciences, bioinorganic chemistry and biological imaging with particular emphasis on:

INORGANIC CHEMISTRY- Design, synthesis and physical properties of coordination complexes, including systems incorporating novel functionality for magnetic resonance and fluorescence imaging of biological systems.

ELECTRON TRANSFER MECHANISMS- Investigate long-range electronic coupling through stacked, π -unsaturated systems, conducting biopolymers and the development of biosensors

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BIOLOGICAL MICROSCOPY- Design and synthesis of spectroscopic and magnetic probes for *in vivo* microscopic imaging of nerve patterning, regulation of cell lineage, gene expression and DNA transfection.

METAL COMPLEXES AS ENZYME INHIBITORS- Investigate the interaction of small-molecule transition metal complexes as enzyme inhibitors for the development of therapeutic antitumor and antiviral drugs.

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Honors and Awards

National Academy of Engineering Lecturer, Cleveland, Ohio, 2000.
Pendergast Lecturer, University of Pennsylvania, 1999.
American College of Neuropharmacology Lecturer, 1999.
Grubstakes Award, Direct Detection of Gene Expression via Magnetic Resonance Imaging; Caltech, 1996, 1998.
Watson Lecturer, Caltech, 1997.
NIH Postdoctoral Research Fellow, 1986.
National Research Service Award, Harvard Medical School, 1985.
Ohio State University Teaching Award (student selected), 1981.
B.S. with Honors Thesis, 1980.

Professional Service

Editorial Advisory Board: Inorganic Chemistry; 1999-
Program Workshop Chair, CTEP drug development program,
National Cancer Institute; 1999-2000.
Editorial Advisory Board: Bioconjugate Chemistry; 1999-.
Ad hoc member; NIH Metallobiochemistry Study Section; 1999-.
Chairmen: Imaging in 2020; National Cancer Institute; 1999
Founder and Scientific Advisory Board Member, Metaprobe LLC
Pasadena, CA. 1998-present.
Chairmen: After The Genome IV Conference; 1998.
Guest Editor: Coordination Chemistry Review, 1998-99.
Cofounder and Scientific Advisory Board Member, Clinical Micro Sensors Inc., Pasadena, CA. 1995-present.
11 named and 73 Invited lectures; 1992-2000.

Teaching

Supervised undergraduate and graduate laboratories and led recitation sections (O.S.U. 1980-81). Ohio State University Teaching Award (student selected) 1981.

Codirector and Founder; "Seeing is Believing: A Classroom Tour of the Sciences." Pasadena Unified School District and local private schools, 1994-present.

Affiliations

American Chemical Society, American Association for the Advancement of Science, International Society for Magnetic Resonance in

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Medicine, Sigma Chi Honorary Research Society, Alpha Chi Sigma,
New York Academy of Sciences, Society for Molecular Imaging.

Trainee History

Supervised 16 undergraduates, 9 graduate students and 16 post-doctoral fellows, 1992-present.

Publications

Meade, T.J., Iyenger, P.A., Frey, P.A., "Synthesis and Rearrangements of Alkyl Phosphorothioates", Journal of Organic Chemistry, 1985, 50, 936.

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